植 物 分 类 与 资 源 学 报 2015, 37 (2): 141~144
Plant Diversity and Resources DOI: 10.7677/ynzwyj201514063

南天竹微卫星标记开发及特性分析*

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摘要: 南天竹 (*Nandina domestica*) 是一种具有较高观赏价值的常绿灌木。本研究从南天竹基因组中开发和筛选出 10 个微卫星位点, 能在该物种中进行稳定 PCR 扩增且具一定的多态性。采用 4 个南天竹群体 24 个个体进行检测发现, 每个位点的等位基因数为 2~6, 期望杂合度和表观杂合度分别为 0.153~0.778 和 0~1。本研究结果为后续对南天竹遗传多样性的研究打下了基础。另外, 这 10 个位点在小檗科 4 个其他物种中也有一定的通用性。

关键词: 南天竹; 微卫星; 遗传多样性
中图分类号: Q 785, Q 16 **文献标志码**: A **文章编号**: 2095-0845(2015)02-141-04

Development and Characterization of Microsatellite Loci for Heavenly Bamboo (*Nandina domestica*)*

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Abstract: We developed and characterized a total of 10 novel polymorphic microsatellite loci from the genome of an ornamental shrub, *Nandina domestica*. The number of alleles per locus in 24 individuals from four populations varied from 2 to 6. The expected and observed heterozygosity ranged from 0.153 to 0.778 and from 0 to 1, respectively. These markers will provide tools for future studies on population genetic diversity of *N. domestica*. Additionally, most of our new markers could yield the amplification products in four other Berberidaceae species.

Key words: *Nandina domestica*; Microsatellite marker; Genetic diversity

Nandina is a monotypic genus in the Barberry family, Berberidaceae. The only species *Nandina domestica* Thunb. (Heavenly bamboo) is a suckering shrub occurring in forest understories of eastern Asia and India (Knox and Wilson, 2006; Ying *et al.*, 2011). It is widely grown in gardens as a beautiful ornamental plant because its young leaves in spring are brightly colored pink to red before turning green, and old leaves turn red or purple before falling. However, in the southeastern U. S. A., this species is considered as a potentially invasive species since it has escaped cultivation in nine states (Knox and Wilson, 2006). Historical and observational data for invasive species are often sparse and incomplete, so molecular genetic markers are increasingly used and have proved to be efficient tools for the inference of invasion origins and routes (Estoup and Guillemaud, 2010). Here we report 10 novel microsatellite loci isolated and characterized from *N. domestica* that can be useful in future studies on the ge-

* Funding: National Natural Science Foundation of China (grant nos. 31160043 and 31160082) and National Science and Technology Support Program (2012BAC11B02)
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Received date: 2014-04-10, Accepted date: 2014-07-20
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netic differences between native and invasive populations, as well as phylogeographic analyses and cultivar identification.

1 Method and result

We sampled 24 *N. domestica* trees in four natural populations from Jiangxi Province (Wuning: 29.1592° N, 115.2608° E; Xinyu: 27.5931° N, 114.5374° E; Taihe: 26.5115° N, 115.0012° E; Yudu: 25.6304°N, 115.3470°E; 6 individuals per population). Voucher specimens for each population were deposited in the Jiangxi Agricultural University herbarium (accession nos. JXAU35229-JXAU35232). Genomic DNA was extracted using the DNeasy Tissue Kit (QIAGEN) and microsatellites were isolated using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane *et al.*, 2002). A single individual of Wuning population was used to prepare the microsatellite-enriched library. Total genomic DNA (*ca.* 250–500 ng) was digested with 2.5 U of MseI restriction enzyme and then ligated to an MseI AFLP adaptor (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′) using T4 DNA ligase (MBI, Fermentas, Lithuania). After ligation the products were amplified with adaptor-specific primers (5′-GATGAGTCCTGAGTAAN-3′/5′-TTACTCAGGACTCATCN-3′). The digested-ligated fragments were diluted in a ratio of 1∶10, and 5 μL of them were used for amplification reactions with adaptor-specific primers (5′-GATGAGTCCTGAGTAAN-3′/5′-TTACTCAGGACTCATCN-3′). The amplified DNA fragments (200–800 bp) were enriched by magnetic bead selection with a 5-biotinylated probe [(AG) 15 or (AC) 15, respectively]. Enriched DNA fragments were reamplified with MseI-N primers. The PCR products were purified using SanPrep Column DNA Gel Extraction Kit (Sangon Bio-Tech, Shanghai, China). Purified DNA fragments were ligated into pGEM-T vector (Promega, Madison, WI, USA), and then transformed into DH5α competent cells (Tiangen Biotech, Bejing, China). The positive clones were tested by PCR using vector primers 5′-CGACTCACTATAGGGAGAGCGGC-3′/ 5′-AAGAACATCGATTTTCCATGGCAG-3′ and primers $(AC)_{10}$/$(AG)_{10}$. A total of 244 positive clones with microsatellite repeats (AG/AC) were identified and sequenced, of which 128 unique clones with relatively long flanking regions were chosen to design primers using Oligo 7.0 software (Rychlik, 2007).

Amplification products and polymorphism of the 128 newly designed primer pairs were assessed with all 24 individuals sampled. The PCR reaction volume (20 μL) contained 50–100 ng genomic DNA, 0.5 μmol·L^{-1} of each primer, and 10 μL 2 × Taq PCR MasterMix (0.1 U Taq polymerase/μL, 0.5 mmol·L^{-1} dNTP each, 20 mmol·L^{-1} Tris-HCl [pH 8.3], 100 mmol·L^{-1} KCl, and 3 mmol·L^{-1} $MgCl_2$; Tiangen). PCR amplifications were conducted under the following conditions: 95 ℃ for 3 min followed by 32–35 cycles at 94 ℃ for 45 s, at the annealing temperature (optimized for each locus, Table 1) for 45 s, 72 ℃ for 45 s; and a final extension step at 72 ℃ for 5 min. PCR products were separated by 8% nondenaturing PAGE gel and stained with a silver-staining method. The experiments were run in duplicate. Estimates of the number of alleles, expected and observed heterozygosity and tests for deviation from both the Hardy-Weinberg equilibrium (HWE) and the linkage equilibrium (LE) were performed with GenePop version 4.0 (Raymond and Rousset, 1995).

Among the 128 primer pairs, 46 were successfully amplified in all samples, and 10 primer pairs displayed polymorphism. Amplified profiles of two microsatellite loci (NTZ01 and NTZ10) on 8% nondenaturant polyacrylamide gel are shown in Fig. 1. Based on 10 polymorphic loci, the number of alleles per locus ranged from 2 to 6. Expected and observed heterozygosity varied from 0.153 to 0.778 and from 0 to 1, respectively (Table 2). All loci were in HWE except NTZ09 and no linkage disequilibrium was detected.

Finally, the transferability of 10 primer pairs was tested in four species from two genera of Berberidaceae.

Table 1　Characteristics of 10 microsatellite primers in *Nandina domestica*

Locus	Primer sequences (5′-3′)	Repeat Motif	T_m /℃	Allele Size /bp	Transferability[a]	GenBank accession no.
NTZ01	F: GGTGTAATAATGGAGAAGG R: ACCCTAAAGTGCCAATGA	$(AG)_8$	57	133	1, 1, 1, 1	KF785778
NTZ02	F: TATACGTGCTTGAGCAATGA R: CAGGCGACAGAAACAGATAA	$(CT)_{12}$	54.5	202	1, 1, 1, 1	KF785779
NTZ03	F: ATGATGATGATGACAGGGA R: TACCACCACCTTCCACAG	$(GA)_{15}$	58.5	267	1, 1, 1, 1	KF785780
NTZ04	F: AAGTGGAGTGAGGCATCTAA R: CTGACAGATGAAGATTACG	$(CA)_7$	51	131	1, 1, 1, 1	KF785781
NTZ05	F: CACTGGATTAGGGCGAGA R: CACCTCCCAGAACTGAAA	$(TA)_{12}(AG)_7$	52.5	121	1, 1, 1, 1	KF785782
NTZ06	F: CTCTTGCCTATTCCCTTC R: GTATGCTATAAGTCGTC	$(CT)_{10}$	45	186	1, 1, 0, 1	KF785783
NTZ07	F: ACAAATAACGAGACTAATCA R: ACCTCGTAAATCTTGTCTTC	$(AG)_7$	48	133	1, 1, 1, 0	KF785784
NTZ08	F: ACCCACAGCCAAACTCCA R: GGTTATCTTATCCCTCTGA	$(AC)_8$	56	168	1, 1, 0, 1	KF785785
NTZ09	F: CTTAGGTTGATTATCTCGG R: GTCAGGCAAGGTTCATGT	$(CA)_7$	55	131	1, 1, 1, 1	KF785786
NTZ10	F: GGCAGTTCGTATAAAGTGG R: GTCATGCAAATCCGTTAG	$(TC)_9$	57	171	0, 1, 0, 0	KF785787

Note: T_m = annealing temperature when run individually. [a] The number 0 or 1 shows the loci could not be or could be amplified successfully in *Mahonia bealei*, *M. japonica*, *Berberis julianae*, and *B. thunbergii*

Table 2　Results of initial primer screening in populations of *Nandina domestica*

Locus	Wuning population (n=6)			Xinyu population (n=6)			Taihe population (n=6)			Yudu population (n=6)		
	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e
NTZ01	2	0.333	0.444	2	0.333	0.444	2	0.000	0.444	2	0.167	0.153
NTZ02	2	0.000	0.278	2	0.167	0.153	2	0.333	0.278	3	0.500	0.569
NTZ03	2	0.333	0.278	2	0.500	0.375	2	0.667	0.444	3	0.333	0.292
NTZ04	3	0.500	0.569	3	0.167	0.486	2	0.833	0.486	3	0.667	0.542
NTZ05	4	1.000	0.681	5	1.000	0.694	3	1.000	0.625	4	1.000	0.736
NTZ06	4	0.667	0.667	4	0.500	0.722	3	0.833	0.611	3	0.500	0.625
NTZ07	2	0.167	0.153	2	0.500	0.486	2	0.000	0.500	2	0.333	0.500
NTZ08	2	1.000	0.500	2	1.000	0.500	3	1.000	0.611	3	0.667	0.500
NTZ09	6	0.667	0.778	6	0.833	0.778	4	0.500	0.653	5	0.667	0.722
NTZ10	3	0.667	0.625	4	0.833	0.681	3	0.500	0.403	3	0.833	0.625

Note: N_a = number of alleles per locus; H_o = observed heterozygosity; H_e expected heterozygosity; n = sample size for each population

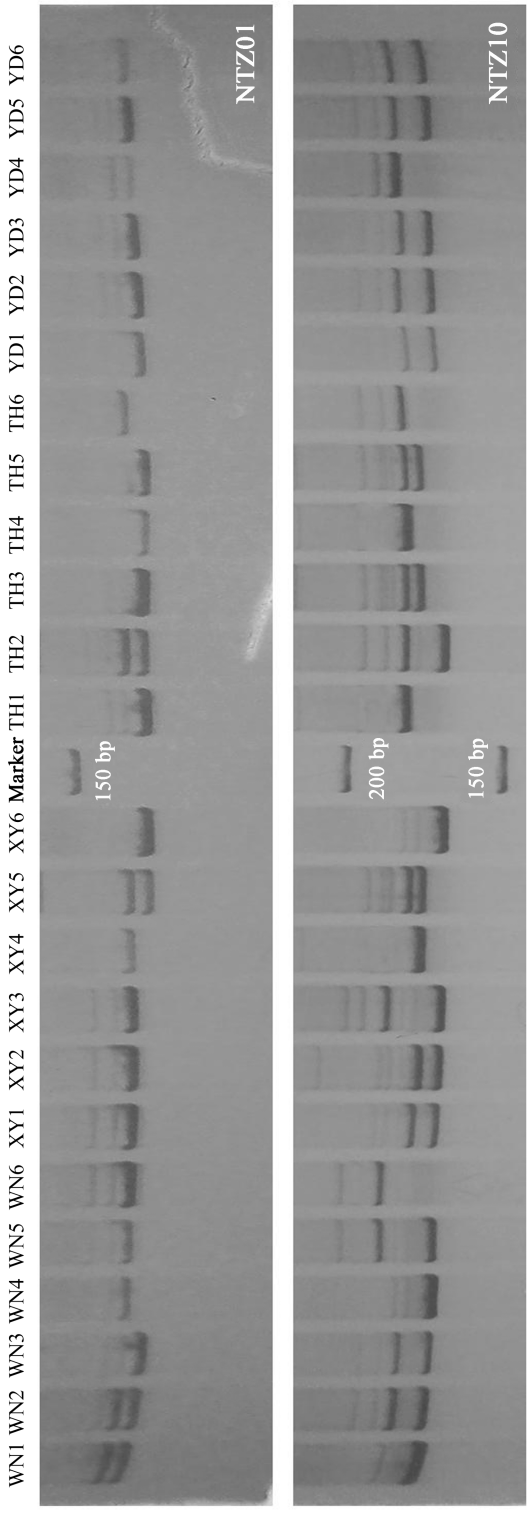

Fig.1 Amplified profiles of two microsatellite loci (NTZ01 and NTZ10) on 8% non-denaturant polyacrylamide gel

Nine, 10, 7, and 8 markers were successfully amplified in *Mahonia bealei*, *Mahonia japonica*, *Berberis julianae*, and *Berberis thunbergii*, respectively (Table 1).

2 Conclusion

The 10 microsatellite markers developed here will be used in our ongoing research on population structure and genetic relatedness in native and invasive populations of *N. domestica*. Such assessment may help researchers reveal the invasion history of the species and rapid evolution within the invasive range. In addition, these markers are also useful for population genetic studies in other genera of Berberidaceae.

Acknowledgments: We thank Mr. Wei Zeng, Tao Li, Jiapeng Zheng and Bochao Li for help with the field survey and leaf collection.

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